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A DISEASE OR TERATOLOGICAL
MALFORMATION OF LUCERNE.



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ART. XIV.—*A Disease or Teratological Malformation of
Lucerne.*

By ELLINOR ARCHER, B.Sc.

(With Plate X.).

(Read December 14th, 1916).

Description of Abnormality

The curious malformation about to be described was found on two specimens of lucerne received from different parts of Victoria, one being from the Werribee Irrigation Settlement, and the other from Echuca. They had both been collected in February, 1916, which was an exceptionally dry month.

The only parts to be affected by the deformity are the flower heads. The main stalks and the leaves are all normal, except a few small leaves at the termination of the main stalk, which show the terminal leaflet very much elongated in proportion to the lateral leaflets. The lower leaves and stem show symptoms of rust, caused by *Uromyces striatus*, but this is hardly likely to have any connection with the malformation.

The place of true flowers has been taken by clusters of small, indefinite heads, which show no special structure until they are examined microscopically.

These heads, as far as could be judged from the dried specimen, were dull grey in colour, with a faint suggestion of the purple characteristic of normal flowers.

If each head is taken to represent a flower, the inflorescence will be found to have increased in complexity from a simple raceme to a raceme of racemes which may be several times compound.

The main axes are normal, but the secondary are somewhat elongated, and bear still smaller axes instead of forming the peduncles of the flowers. The bracts to the main and secondary axes are normal.

Microscopic examination of the heads shows these axes to be terminated by masses of rounded growing points, which are enveloped by what may be described as small, narrow, simple bracteate leaves. Apparently all the growing points ultimately develop into these abnormal leaves, there being no true flowers or rudiments of parts of flowers to be found on either specimen.

The actual growing points show no special feature, being simply rounded masses of meristematic cells, with large granular nuclei. Careful examination of the bracteate leaves shows them to be small structures not more than two or three layers of cells thick, and varying very much in length, those placed lowest on the axis being the longest.

A rudimentary vascular strand can be found in all, and in the largest a few spiral and annular vessels are developed. Transverse section shows no distinction into palisade parenchyma and spongy mesophyll, but the internal cells are differentiated from the epidermal by being smaller and having thinner walls. A few of them terminate in a curious unicellular, hair-like projection. The larger bracts show a few stomata, but the most noticeable feature is the extensive deposit of calcium oxalate crystals along the vascular strand. These crystals occur in the same form along the mid-rib of normal foliage leaves, but as the bracts are so much smaller the crystals appear to be more prominent.

It would be impossible to say whether these bracts represent abortive foliage or floral organs. From their position and suggestion of colour they seem to represent the floral whorls. They are indefinite in size, number and position, but they appear to wrap round and protect the young growing points.

Normal hairs occur at the base of a cluster of heads and bracts. These hairs have not suffered in the general abortion, and consequently appear rather large in comparison with the rest of the heads.

Sections.

Microtome sections of the material were made, and carefully stained and examined. Some of the stains used were methyl, blue, fuschin, safranin and gentian violet.

A careful examination made for any sign of parasitic fungi gave a negative result. If there had been any hypha present these should have been clearly stained by the methyl blue or safranin. No hypha could be seen, and the cells showed no sign of disintegration. Staining with concentrated alcoholic solution of Fuschin revealed numerous granules, varying very much in size. These were embedded in the cell walls or lining layers of protoplasm. They were most numerous in sections passing through the phloem of vascular bundle of the stems; very few could be distinguished in the very young growing points or the younger bracts.

High-power examination with an oil immersion lens did not reveal any structure in these granules. The sections were tested with iodine for starch, but the granules gave a negative reaction. They were probably proteid in character, as similar granules which have been proved to be proteid have been found in other plants. Further investigation is needed to make sure of this.

Sections of normal lucerne, which were subjected to the same embedding and staining processes, showed no trace of the granules, but here again more work must be done before the evidence is entirely satisfactory.

Staining with gentian-violet showed the cortical cells, abnormal stem and bracteate leaves to be densely packed with small, rod-like structures. These did not stain themselves, but showed clearly in the coloured protoplasm. They could be seen to be actually embedded in the protoplasm of the cell, and were not in the cell sap.

These gave a good positive reaction with chlorozone iodine, and a faint blue with strong solution of iodine in potassium iodide, which proves them to be an unusual form of young starch grains, or ones in which the cellulosic basis is more prominent than the granulose. They were especially numerous in sections of leaves from the abnormal plant.

Gram's special staining method to indicate the presence of bacteria in cells gave a negative result.

Culture Solutions.

The only growth which could be definitely traced as originating from the lucerne was the fungus *Macrosporium*, and this was found to develop from both specimens.

Very careful examination of the dried material showed the presence of *macrosporium* hyphae on the bracts themselves.

As *macrosporium* is usually a superficial fungus, and as it has been proved that there is no endophytic fungus present, it is very unlikely that it is the primary cause of the malformation.

Cause of Malformation.

As far as the investigation has gone at present it would be impossible to state definitely the cause of the abnormality, but it is possible to exclude some factors which might have affected the plant.

1. The malformation does not appear due to parasitic fungi.
2. There is no indication of the presence of destructive

insects, the bites of which might very easily have caused the proliferation of growing points.

3. The tissues do not seem to be infected with bacteria, and the cells do not show abnormality of size or shape, which is usually the case in the presence of bacteria.

The abnormal starch grains and proteid granules point to abnormal nutrition, and the fact that both specimens came from dry country, and were produced during a drought period, may possibly have something to do with the cause.

DESCRIPTION OF PLATE X.

- Fig. 1.—Stem leaf and inflorescence of abnormal plant.
- „ 2.—Inflorescence of normal lucerne (*Medicago sativa*).
- „ 3.—Head of abnormal material, showing growing points and bracteate leaves (magnified low power).
- „ 4.—Section of growing points and bracteate leaves (magnified low power).
- „ 5.—Single bracteate leaves (magnified high power).
- „ 6.—Section showing proteid granules (magnified high power).
- „ 7.—Section showing starch grains (magnified high power).

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